homozygous. If the target DNA is heterozygous, the beads binding across the SNP site will consist of two populations with either a C or an A component at the SNP position. If the DNA used as a template for the amplification of the SNP regions is pooled from numerous sources, the relative frequency in the population of the different polymorphisms at a given SNP position will be reflected in the relative abundance of the amplification products. The relative polymorphism frequency will therefore also be reflected in the binding signal intensity ratios of the different bead sequences that span the SNP site. Numerous polymorphic sites in a large genome can be analyzed simultaneously by amplifying and sequencing short regions from the genome that incorporate the polymorphisms. For example, the 50 nucleotides flanking an SNP require approximately 40-50 distinct beads for analysis. Since this approach is equivalent to DNA sequencing, the library capacity constraints are similar. A million bead library of 10-mers can therefore analyze the relative frequency of a few tens of 50 base SNP fragments. If 12-mers are employed, the library size grows to 16 million, enabling the detailed analysis of roughly 100 SNPs.

An alternative means for analyzing SNPs is to employ polymorphically selective amplification during the production of the target DNA. That is, if a specific polymorphism is present, it will be amplified from the SNP region. Absence of the polymorphism results in no amplification. Because the polymorphic selectivity is inherent in the amplification process, the bead analysis serves only to identify the presence or absence of a reaction product. The presence of a reaction product can be determined by a binding signal on as few as one unique bead associated with the product, which is a far more relaxed constraint on the bead library's analytical capacity than the case of DNA sequencing and greatly increases the analytical capacity. For example, if 50 base stretches of DNA are amplified from multiple SNP sites and pooled for analysis, each SNP fragment must differ from all the others by at least one bead. Hence, there can be extensive cross-branching of the SNP fragment hybridization patterns. The analytical capacity of a library therefore approaches the size of the library divided by the number of beads needed to hybridize to a given SNP fragment. In the case of a million bead 10-mer library with 50 base fragments, on the order of 10,000 SNPs can be analyzed simultaneously.

A variation on the above method of SNP genotyping is to employ a different probe color for each base that may be present at an SNP site. In this manner, relative polymorphism abundance can be obtained without the need to sequence the SNP regions.

In addition to DNA sequencing and polymorphism analysis, flow imaging of bead/oligo libraries can be used to analyze gene expression. In the case of gene

expression analysis, specific sequences from each of the genes of interest are extracted from RNA. Extraction can take the form of splicing out and labeling sections of RNA itself or converting the RNA to labeled DNA, for example by reverse transcriptase PCR. The same basic principle of the second method of polymorphism analysis applies to expression analysis, namely that there is no need to sequence the entire expressed gene. Instead, the presence of a specific fragment, as indicated by the binding of at least one unique bead to the amplified expression sequence, indicates the expression of a particular gene. As in sequencing polymorphic sites, the intensity of the binding signal can indicate the level of expression.

The hybridization of DNA to bead-bound oligos is reversible, raising the possibility that a bead library could be used a number of times. There are several ways to restrict the reuse of a bead library based on the fact that the correspondence between each bead signature and its associated oligonucleotide sequence is determined and controlled during the production of a bead lot and can be varied from lot to lot. The information necessary to decode oligo sequences from the bead signatures detected during analysis can be restricted to prevent the unauthorized use of a bead library. The analysis platform could identify the unique lot number of the bead library via a barcode or other identifier and employ a corresponding decoding file distributed by the authorizing body to perform the analysis once, or a given number of times. Another method of restricting authorization might require the user's analytical platform to contact the authorizing entity with the bead lot number at the time of analysis, via the Internet for example, whereupon the identity and validity of the user is determined. Alternately, the beads could be physically altered during analysis, for example by illumination with 260 nm light, to destroy the bound oligonucleotides and prevent reuse.

The use of reporter labeled libraries in flow imaging is not limited to DNA and RNA analysis. Any molecule that can be synthesized on a bead, such as amino acids or drug candidates, can be encoded and read in flow. The concept can even be extended to living cells. Cells of different types can be encoded by attaching labels to their membranes or locating those labels within the cell itself. For example, a reporter with a unique signature identifying a corresponding specific receptor-specific antibody could target any cells expressing that receptor on the cell surface. In some cases, subsequent endocytosis of the receptor-antibody complex would then internalize the reporter bead as well. This binding would identify those cells expressing a specific receptor. Reporters could be internalized by the cell via a variety of mechanisms, including, but not limited to, phagocytosis, endocytosis, lipophilic conjugation, protein transduction, or alternatively through pores created in the cell membrane via exposure to toxins, such as streptolysin O,tetanolysin, E. coli hemolysin, via electroporation, or using other

means known to one skilled in the art. Specific reporters can be used to indicate cell type, added over time to indicate cell age, and or incorporated upon the cell's exposure to certain drugs or environmental conditions. For encoding even greater diversity of cell types or cell processes, the concept can be extended to tagging cells with reporter labeled carrier assemblies. In this case, the cell acts as a carrier bead which carries other reporter labeled carrier beads. Note also reporter labeled compounds are not limited to multi-component compounds being labeled as there are synthesized. Single component and already synthesized multi component compounds can be bound to beads. Reporters can then be used to label any such compound associated with a bead.

Referring once again to FIGURE 22, a flowchart 350 provides the series of logical steps employed to generate and use an encoded bead library for either genotyping, sequencing, or expression profiling. In a block 352, a library of N-mer oligos is generated. The combinatory SAP scheme described above can be beneficially employed, as well as a directed synthesis. While most of the current discussion has been directed to optical reporters that use color as a distinguishing characteristic, it should be noted that other reporters, with other distinguishing characteristics, as noted above, can also be beneficially employed to generate an encoded bead library of the desired size. In a decision block 354, a user must select the type of analysis to be performed (genotyping, sequencing, or expression profiling).

If genotyping is selected, the logic proceeds to a block 356, and the genomic DNA is amplified using primers for polymorphic regions of interest, as discussed above. If in decision block 354, expression profiling was selected as the analysis of choice, then in a block 358, RNA is amplified using primers for genes of interest, also as generally discussed above. Alternatively, if sequencing is selected in decision block 354, then in a block 360, a user would amplify genomic DNA using primer for extended sequences of interest.

Regardless of the analysis selected, the next step is to hybridize the amplified DNA/RNA to the bead library in a block 362. In a block 364, the beads are analyzed, preferably by employing one of the flow imaging systems discussed above, to identify oligo sequences of positive beads. Once the positive beads are imaged, the final step is to construct sequence contigs to identify genomic DNA sequences, polymorphic alleles, or expressed genes, in a block 366.

Although the present invention has been described in connection with the preferred form of practicing it and modifications thereto, those of ordinary skill in the art will understand that many additional modifications can be made to the present invention within the scope of the claims that follow. Accordingly, it is not intended that